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Qualitative and Quantitative Parameters Analyses of Buffalo Ovaries

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ABSTRACT

Buffalo ovaries were collected from abattoirs in Dhaka city. During collection, left and right ovaries were identified and recorded. After processing, the ovaries were categorized as (i) ovaries without corpus luteum (CL) and (ii) ovaries with corpus luteum (CL). Ovaries were then evaluated based on length (cm), width (cm), weight (g), total number of follicles on the surface of each category of ovaries, number of follicles aspirated, and total number of cumulus-oocyte-complexes (COCs). The experiment's findings indicated variations in the parameters related to the left and right ovarian categories. The CL% (27.12±0.11%) found highest in the right compared to the left (3.24±0.11%). The length (cm), width (cm), and weight (g) (2.75±0.06, 2.17±0.05, 4.74±0.13) were found significantly ($p<0.01$) higher in the right ovaries compared to left (2.27±0.06, 1.77±0.05, 3.90±0.13). The number of follicles in total and number of follicles aspirated were found significantly ($p<0.01$) higher (6.35±0.16 & 5.47±0.16) in the left ovaries compared to right (6.16±0.16 & 4.18±0.16). The number of normal COCs and total COCs was significantly ($p<0.01$) higher in the left ovaries (0.98±0.134 & 1.57±0.17) compared to right (0.84±0.16 & 1.54±0.17). The abnormal COCs were significantly ($p<0.01$) higher in the right ovaries (0.75±0.107) compared to left (0.57±0.107). Regarding ovarian categories; (76.47±0.01%) showed ovaries without CL, whereas (23.53±0.01%) showed ovaries with CL. The weight (g) was found significantly ($p<0.01$) higher in ovaries with CL (4.73±0.12) compared to ovaries without CL (4.36±0.12). The number of follicles in total and aspirated were found significantly ($p<0.01$) higher in ovaries without CL (7.60±0.14 & 5.28 ±0.14) compared to ovaries with CL (4.29±0.14 & 2.29±0.14). Regarding the number of total COCs, ovaries without CL showed a significantly ($p<0.01$) higher number of total COCs (1.41±0.11) compared to ovaries with CL (1.17±0.11), where the number of normal and abnormal COCs (0.55±0.01 & 0.86±0.03) were found significantly ($p<0.01$) higher in ovary without CL compared to the ovary with CL (0.5±0.01 & 0.54±0.03). Finally, it can be concluded that the left and ovaries without CL contain more follicles in total and aspirated and normal COCs. Thus, the left and ovaries without CL are best for collecting quality COCs in buffaloes.

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INTRODUCTION

Buffalo (Bubalus bubalis) is an integral part of the agricultural economy and plays important role in livestock production. Asian buffalo or Water buffalo is classified under the genus *Bubalus,* species *bubalis.* Bangladesh now has about 1485000 buffaloes for draught or dairy (BBS, 2018). These buffalo are found in the Brahmaputra-Jamuna flood plain of central Bangladesh, the Ganges-Meghna flood plain of southern Bangladesh, and institutional herds. Bangladesh has milk/dairy buffaloes of the Swamp crossbred and River types such as the Murrah and Nili-Ravi. The occurrence of crossbred dairy buffaloes indicates that the genetic improvement program has been operative and is still running (Faruque, 2000). Reproductive efficiency is the primary factor affecting productivity. It is hampered in female buffalo by (1) inherent late maturity, (2) poor estrous expression in summer, (3) distinct seasonal reproductive patterns, and (4) prolonged calving intervals. Reproductive efficiency can be improved by introducing embryos produced in vitro (Raza et al*.,* 2001). Oocytes are the main raw materials for in vitro embryo production (IVP) experiments. Though a lot of ovaries are wasted in slaughterhouses it may be a good source of quality livestock production that can fulfill the existing scarcity of meat, milk, and skin. (Asad et al., 2016). Ovaries were categorized as right and left, with corpus luteum (CL) and without CL group. Ovaries were then evaluated based on weight (gm), length (cm), width (cm), total number of follicles on the surface of each categorized ovary, number of follicles aspirated, total number of COCs, normal COCs, and abnormal COCs. The oocytes from ovaries without CL had greater developmental competence than ovaries with CL (Tasripoo et al*.,* 2005). Over a decade, there was much research done towards the implementation of embryo technologies to fasten the genetic manipulation of livestock, which involves multiple ovulation and embryo transfer (MOET), in vitro embryo production (IVP), cloning, and transgenesis (Asad, 2015; *Saha* et al., 2014; Sreenivas et al*.,* 2014; Freitas and Melo, 2010). From those mentioned, IVP has become a more popular method of producing embryos from slaughterhouse-derived ovaries (Hoque et al*.,* 2012). The IVEP system involves at least four steps, namely (i) evaluation of ovaries, efficient collection and grading of oocytes; (ii) in vitro maturation (IVM) of these oocytes; (iii) in vitro fertilization (IVF) of the matured oocytes; and (iv) in vitro culture (IVC) of the resulting embryos (Freitas and Melo, 2010). In Bangladesh, in vitro techniques in buffalo is a recent concept, but a great deal of work is still going on to standardize IVP techniques followed by IVM and IVF (Ferdous, 2006; Islam et al*.,* 2007; Mondal et al*.,* 2008; Hoque, 2009). A critical goal for the mass production of buffalo embryos is the recovery of many oocytes with high developmental competence. Moreover, successful embryo production also depends on oocyte quality. The objectives of this experiment are to evaluate the ovaries based on the recovery of follicles and oocyte analysis and to categorize the ovaries according to the number of follicles and oocytes used for the in vitro production of embryos.

MATERIALS AND METHODS

The present investigation on “Ovarian categories, follicles and oocytes analysis of buffalo in viewof *in vitro* production of embryos” was conducted from January 2018 to December 2018 at the Animal Nutrition, Genetics and Breeding Laboratory at Sher-e-Bangla Agricultural University, Dhaka-1207.

Chemicals and culture media

All media and chemicals from M/S Sigma Aldrich Chemicals Co. (St. Lois, MO, USA) and HiMedia Laboratories Private Limited were used unless otherwise mentioned. 0.22 µm and 0.45 µm filters, disposable petridish (large 90 mm, small 35 mm diameter), and 15 ml graduated tubes of the Axiva brand were used.

Preparation of different media

The stock solutions used in the study were prepared using milli Q water (Integral 5, Millipore). All the working solutions/media, excluding Oocyte Collection Medium (OCM), were kept for 3-4 hours in a CO_2 incubator for quenching before use. The stock of all media was stored at 4°C and used within fifteen days of preparations. All the culture media were sterilized by filtration througha 0.22 µm filter stored at 4°C and used within one week.

Collection and Processing of ovaries

From nearby slaughterhouses, buffalo ovaries with unknown reproductive history were collected. The ovaries were stored in a collection vial with 0.9% physiological saline in a thermos flask at 25–30°C for 4–5 hours. On the collection day, 5 lac IU of penicillin and 100 mg of streptomycin were administered per liter of saline. The ovaries were then moved to the sterilized petridishes with the same saline, completely cleaned with a physiological solution at room temperature, and labeled as the left and right ovaries (a butcher assisted in tagging the ovaries after slaughter). The presence or absence of the corpus luteum (CL) was also noted.

Evaluation of ovary

Measurement of length, width, and weight

The length and width of the ovaries (right and left ovaries; ovaries with CL and ovaries without CL) were measured with slide calipers and expressed in cm. The weight of individual ovaries was measured by placing them on a digital balance and recorded in tabular form.

Follicles counting on the surface of the ovary

There are many follicles on the surface of both ovaries. The number of visible follicles on the surface of different categories of ovaries was counted and recorded accordingly. The highest number of visible follicles was 8, whereas the lowest was 2.

COCs aspiration and grading

The ovaries were washed 2-3 times in saline solution at room temperature. They were then placedin a beaker and kept in a water bath at 300C. One ovary was picked up in hand. The 10 ml syringewas loaded with 1.0-1.5ml of PBS (Sigma, USA), and the needle (18G) was put in the ovary parenchyma near the vesicular follicles of 2-6 mm diameter, and all follicles were aspirated near the point. After aspirating the follicles from one ovary, the aspirated follicular materials were transferred slowly into 90-mm petridishes, avoiding damage to the cumulus cells, and the (cumulus-oocyte-complexes) COCs were searched and graded under the microscope at low magnification. The COCs were then classified into 4 grades according to the slight modification of the method of Khandoker et al. (2001), where Grade A: oocytes surrounded by cumulus cells; Grade B: oocytes partially surrounded by cumulus cells; Grade C: oocytes not surrounded by cumulus cells and Grade D: degeneration observed both in oocytes and cumulus cells. Grades A and B were considered normal COCs, and grades C and D were considered abnormal. In the meantime, another Petridishes of D-PBS was prepared for pooling COCs, and the COCs were picked up with an appropriate glass micropipette. The tip diameter of the pipette was checked under the microscope to ensure COCs could be easily aspirated without damaging the cumulus cells. Then the COCs were washed 2-3 times into D-PBS.

Statistical analysis

The data on various aspects with and without CL, viz., ovarian weights, follicular counts, oocyte retrieval rate, oocyte recovery rate, with different oocytes collection techniques, viz., oocyte retrieval rate, oocyte recovery rate, and grading of oocytes were suitably tabulated and analyzed using SAS statistics software. The differences among the parameter means were performed using DNMRT (Duncan's New Multiple Range Test).

RESULTS AND DISCUSSION

Buffalo ovaries were collected from different abattoirs of Dhaka city, left and right ovaries were recorded and tagged. The ovaries were then classified into two types: the ovaries without corpus luteum (CL), which is in the follicular phase, and the ovaries with CL, which is in the luteal phase. Among 102 ovaries (51 left and 51 right), CL presents 27.12% in the right and 3.24% in the left ovaries. The average length, width, weight, and number of follicles observed and aspirated and collected COCs from left and right ovaries are summarized in Tables 1 to 6.

Ovarian categories regarding left and right category

With or without corpus luteum (CL)

Significant variation was found on the ovary with corpus luteum (CL) or without corpus luteum (CL) in both the left and right ovaries. Results showed that the highest percentage of CL (27.12%) was found in the right ovary, whereas the lowest percentage of CL (3.24%) was found in the left ovary. Similarly, the highest percentage of CL (94.76%) was found in the left ovary, whereas the lowest percentage of CL (72.88%) was found in the right ovary. Follicular growth initiation is one of the most critical and least understood aspects of ovarian biology and represents a significant challenge for experimental study. Changes in the local microenvironment, such as the pH and hormonal concentration, probably occur as the follicles evolve into the primary stage, but these were probably affects rather than causes (Webb et al., 1999). Due to the absence of CL in the without CL group the negative effect of progesterone on anterior pituitary might not be functional in this ovary. So, the highest number of COCs in this category, other than the CL functional group, explains the role of hormonal balance on buffalo folliculogenesis. Within the category, the higher number of normal COCs than that of abnormal COCs further supports the above statement. The CL-absent group ovaries explain the role of progesterone on buffalo follicular degeneration.

Length of ovary (cm)

Among different parameters obtained from different categories of ovaries, the mean length (cm) was significantly varied between left and right ovaries (Table 1). The mean length (cm) was significantly ($p<0.01$) higher in the case of the right ovaries (2.75±0.056) compared to the left ovaries (2.27±0.056), which is almost similar to the previous study by Islam *et al*. (2007). The collected goat ovaries were categorized as right and left, with corpus luteum (CL) and without CL group. It was also categorized based on length (cm), width (cm), and weight (gm). The length (cm) of the right ovaries(1.19±0.09) was found to be significantly ($p<0.05$) higher than that of the left ones (1.15±0.04), which is almost similar to the previous study of Asad et al. (2016).

Width of ovary (cm)

The width of the ovary was obtained from different categories of ovaries (left and right), and the mean width (cm) varied significantly (Table 1). The mean width obtained was significantly ($p<0.01$) higher in the right ovaries (2.17±0.051), whereas the lowest was found in the left ovaries (1.77±0.051). Collected buffalo ovaries from a slaughterhouse and transported them to the laboratory in saline solution at 36°C. The means of weight, length, width, and height of the ovary were 3.83 g (n=84), 2.27 cm (n=84), 1.08 cm (n=84), and 1.56 cm (n=84), respectively, which supports the previous study of Leal et al. (2007).

Table 1. Ovarian categories regarding length, width, and weight of ovary

Ovarian categories	Length (cm) (Mean ± SE)	Width (cm) (Mean ± SE)	Weight (g) (Mean ± SE)
Left (51)	2.27^{a} ± 0.06	1.77^{a} ± 0.05	3.90^{a} ± 0.13
Right (51)	2.75^{b} ± 0.06	2.17^{b} ± 0.05	4.74^{b} ± 0.13
CV (%)	16.03	18.33	21.61

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

Table 2. Ovarian categories regarding on number of follicles in total and aspirated

Ovarian categories	Number of follicles	
	Total (Mean ± SE)	Aspirated (Mean ± SE)
Left	6.35^{a} ± 0.16	5.47^{a} ± 0.16
Right	6.16^{b} ± 0.16	4.18^{b} ± 0.16
CV (%)	26.88	34.61

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

Weight of ovary (g)

Significant variation was found in the weight of ovaries obtained from different categories of ovaries (left and right) (Table 1). Results indicated that the mean weight (g) found was significantly ($p<0.01$) higher in the right ovaries (4.74±0.13), whereas the lowest was found in the left ovaries (3.90±0.13). A study on 50 native buffaloes of Odisha to evaluate ovarian biometry found insignificantly higher average weight (g) of the right ovary (2.36±0.13) than the left ovary (2.17±0.11) conducted by Patra et al. (2013). Among 136 ovaries (68 on each side, i.e., left and right), 93 belonged without CL and others with CL. A non-significant ($P<0.05$) difference was found in the weight of left (2.87±0.32g) and right ovaries (3.59±0.31g) recorded in the weight of buffalo ovaries by Khandoker et al. (2011).

Number of follicles (Total and Aspirated)

Variation in the number of follicles (total and aspirated) was significant ($P<0.05$) regarding follicle count in the left and right ovaries (Table 2). The number of follicles in total and the number of follicles aspirated were observed to be significantly ($P<0.05$) higher in the left ovary (6.35±0.16) and 5.47±0.16), respectively. Again, the number of follicles in total and the number of follicles aspirated were found to be lowest in the right ovary (6.16±0.16 and 4.18±0.16), respectively. The mean count of different-sized follicles in the right ovary and their total count was slightly higher numerically thanthe left ovary, which was statistically non-significant in buffaloes. The average number of large, medium, and small follicles of the right ovary was recorded to be (0.70±0.07,

1.60±0.12, and 6.26±0.37) respectively, with a total of (8.54±0.42); similarly, the left ovary possessed (0.68±0.07, 1.52±0.13 and 6.10±0.32) respectively large, medium and small follicleswith a total of 8.30 ± 0.63 was reported from the study of Patra et al. (2013).

Grading of COCs

Grading of COCs was done in two types as normal and abnormal. Total COCs with normal and abnormal were significant in the left and right ovaries (Table 3). It was observed that normal COCs were higher than abnormal COCs both in the left and right ovaries. Results revealed that normal COCs (0.98±0.13) were found significantly (P<0.05) higher in the left ovary, whereasthe lowest normal COCs (0.84±0.13) were observed in the right ovary. Similarly, the abnormal (0.75±0.11) was found significantly (P<0.05) higher in the right ovary, whereas the lowest abnormal COCs (0.57±0.11) were observed in the left ovary. Regarding total COCs, it was found significantly (P<0.05) higher in the left ovary (1.57±0.17), whereas the lowest result was observed in the right ovary (1.54±0.17). When the COCs were classified into normal and abnormal groups, the highest numbers of normal COCs were found in the left rather than the right ovary, which supports the previous result of Islam (2007). There was a significantly higher (P<0.05) mean number of buffalo oocytes per ovary of grade A (0.82± 0.04) and B (0.79 ± 0.04) followed by grade D (0.76 ± 0.05) than grade C (0.64 ± 0.04) oocytes in slicing method which belongs to the previous study of Makwana *et al*. (2012).

Table 3. Ovarian categories on grading of COCs

Ovarian categories	Grading of COCs		
	Normal (Mean ± SE)	**Abnormal** (Mean ± SE)	**Total** (Mean ± SE)
Left	0.98^{a} ± 0.134	0.57^{a} ± 0.107	1.57^{a} ± 0.17
Right	0.84^{b} ± 0.134	0.75^{b} ± 0.107	1.54^{b} ± 0.17
CV (%)	105.04	116.14	77.81

Means with different superscripts differ significantly from each other within the same column(p<0.01)

Ovarian categories regarding with CL or without CL

Ovary with CL or without CL

The smaller number of CL present group ovaries was found in this experiment because less reproductive performer buffaloes are usually slaughtered, and most of them might be non-cyclic. So, there was the possibility of getting more non-cyclic ovaries from the slaughterhouse during random sampling. Significant variation was found in the corpus luteum (CL) regarding presence or absence. Results showed that the ovary with CL was (23.5±0.01)%, whereas the ovary without CL was (76.47±0.01)%. The causes of more follicles found in ovaries without CL than those of the containing group were understood well as they fit the endocrinological explanation. Various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yielded a significantly higher number of oocytes than luteal phase ovaries. The collected goat ovaries werecategorized as right and left, with corpus luteum (CL) and without CL group. When comparing the ovaries in with CL and without CL group, a significantly (p<0.05) higher number of normal COCs (1.12±0.07) were found in the without CL group with an increase of length (1.17±0.01), which was almost similar to the previous study of Asad et al. (2016).

Length of ovary (cm)

Among different parameters obtained from different categories of ovaries, the mean length (cm) did not significantly vary between ovaries with CL or without CL (Table 4). However, the mean length was higher in the case of the ovary with CL (2.86±0.10) compared to the ovary without CL (2.82±0.10). During the luteal phase, the ovaries had significantly higher values for weight, volume, length, and breadth but not for width. The ovaries in buffaloes aged 3.5 to 7 years were found to be heavier and larger than those of the older animals, probably due to the higher ovarian activity in younger animals, which was found in the previous study of Neelam and Saigal (2005).

Table 4. Ovarian categories regarding with CL or without CL on length, width, and weight

Ovarian categories	Length (cm) (Mean ±SE)	Width (cm) (Mean ±SE)	Weight (g) (Mean ±SE)
With CL	2.86 ± 0.10	2.30 ± 0.01	4.73^{a} ± 0.12
Without CL	2.82 ± 0.10	2.30 ± 0.01	4.36^{b} ± 0.12
CV (%)	18.56	8.78	23.54

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

Width of ovary (cm)

The width of the ovary is obtained from different categories of ovaries (with CL or without CL). The mean width (cm) did not vary ($p<0.01$) significantly (Table 4). The mean width of both ovaries with CL or without CL was same (2.30±0.01) cm. From a study of collected goat ovaries, categorized as right, left, with corpus luteum (CL) and without CL group. It is also categorized based on weight (gm), length (cm), and width (cm). The length (cm) of the right ovaries (1.19±0.09) was found to be significantly ($p<0.05$) higher than the left ones (1.15±0.04) and other parameters, including width, weight, and total number of COCs aspirated per ovary, did not differ significantly ($P<0.05$) between right and left ovaries, almost similar to Asad et al. (2016).

Weight of ovary (g)

Significant variation was found in the weight of the ovaries obtained from different categories of ovaries (with CL or without CL) (Table 4). Results indicated that the mean weight was observed significantly ($P<0.01$) higher in the ovary with CL (4.73±0.12) compared to ovary without CL (4.36±0.12). Among 136 ovaries (68 on each side, i.e., left and right), 93 belonged without CL and others with CL. A non-significant ($P<0.05$) difference was found in the weight of the left (2.87 ± 0.32 g) and right ovaries (3.59 ± 0.31 g); the weight was significantly ($P<0.05$) higher in ovaries with CL (3.64 ± 0.18 g) than ovaries without CL (2.73±0.12 g), which was almost similar to the study of Khandoker et al. (2011).

Number of follicles (Total and Aspirated)

Variation in the number of follicles (total and aspirated) was significant regarding follicle count with CL or without CL (Table 5). The number of follicles in total and number of follicles aspirated was found in the without CL ovary (7.60±0.14) with the highest aspirated follicles (5.28±0.14). Again, the lowest total follicle count was found in CL's present ovary (4.29±0.14) with the lowest aspirated follicles (2.29±0.14). A study reported that the presence of a CL stimulates the development of a significantly higher ($P<0.01$) number of ovarian follicles, which produced a significantly higher ($P<0.05$) number of good-quality oocytes by Abdoon and Kandil (2001).

Collected cow ovaries immediately after slaughter and divided into three categories based on their cyclic status, which included the presence of a large follicle (LF), the presence of a corpus luteum (CL), and ovaries without LF or CL (WLCF). The highest average oocytes collected per ovary were related to the CL (22), WLCF (21.4), and LF groups (20.8), respectively, found in the previous study of Pirestani et al. (2011). Changes in the local microenvironment, such as the pH and hormonal concentration, probably occur as the follicles evolve into the primary stage, but these are probably effects rather than causes (Webb et al., 1999). Growth initiated of follicles has variously been attributed to hormonal triggers (gonadotropins), chiastic process (fluctuation in the internal signal follicle), and external inhibitory control of growing follicles (Webb et al., 1999). The balance between gonadotropins (FSH and LH) and steroids (estrogen and progesterone) might be the essential criteria in this process. The highest number of follicles found in ovaries without CL in the present study might reflect the optimum level of gonadotropins and steroids. This result was comparable with the observation of Wang et al. (2007), who harvested oocytes from the ovary by aspiration (2.9) collection techniques. It also supports the findings of Singh et al. (2013) on goats.

Table 5. Ovarian categories regarding with or without CL on the number of follicles in total and aspirated

Ovarian categories	Number of follicles	
	Total (Mean ± SE)	**Aspirated (Mean ± SE)**
With CL	$4.29^{a} \pm 0.14$	$2.29^{a} \pm 0.14$
Without CL	$7.60^{b} \pm 0.14$	$5.28^{b} \pm 0.14$
CV (%)	28.27	25.44

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

Grading of COC's

The presence of total COCs with abnormal COCs was significant with CL or without CL ovaries, whereas normal COCs were not significant (Table 6). It was observed that normal COCs were lower than abnormal COCs in both with CL or without CL ovaries. Results revealed that the normal COCs (0.55±0.01) were found highest in ovaries with CL, whereas the lowest normal COCs (0.50±0.01) were observed in ovaries without CL. The total number of COCs (1.41±0.11) and number of abnormal COCs (0.86±0.03) were found to be significantly ($p<0.01$) higher in ovaries without CL, whereas the lowest abnormal COCs (1.17±0.11) were observed in ovaries without CL.

Table 6. Ovarian categories of buffaloes regarding with CL or without CL on grading of COCs

Ovarian categories	Grading of COCs		
	Normal (Mean ± SE)	**Abnormal (Mean ± SE)**	**Total (Mean ± SE)**
With CL	0.50 ± 0.01	$0.54^{a} \pm 0.03$	$1.17^{a} \pm 0.11$
Without CL	0.55 ± 0.01	$0.86^{b} \pm 0.03$	$1.41^{b} \pm 0.11$
CV (%)	37.55	42.56	52.66

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

In terms of total COCs, the highest (1.41±0.108) was found without CL ovary, whereas the lowest (1.17±0.108) was observed in with CL ovary. When the COCs were classified into normal and abnormal groups, the highest numbers of normal COCs were found in those without CL ovaries than with CL ovaries. When the COCs were classified into normal and abnormal groups, the highest numbers of normal COCs were found in the left rather than the right ovary, which is almost similar to the previous result of Islam (2007). Age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, and method of oocyte retrieval are some of the factors that might contribute to be recorded variation in oocyte quality (Nandi et al., 2001; Zoheir et al*.,* 2007; Amer et al*.,* 2008). In terms of the quality of oocytes, Ferdous (2006) reported that the numbers of normal COCs were found to be significantly higher ($p < 0.05$) in 2 to 6 mm diameter follicles than others. In the ovaries without CL, the negative effect of progesterone on the anterior was not functional in these types of ovaries.

CONCLUSION

In conclusion, from this research, it is clear that left ovaries contain more follicles and COCs than right ovaries and more normal COCs. Whereas without CL, ovaries contain a higher number of follicles and normal COCs compared to ovaries with CL. So, more normal COCs were found in the left and without CL ovaries. These findings suggest that ovaries without CL and left ovaries are best for optimal oocyte recovery.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflict of interest.

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